Efeito do concentrado protéico de soro e das bactérias ácido láticas endógenas em queijo Coalho com baixo teor de gordura

Effect of whey protein concentrate and endogenous lactic acid bactéria in low-fat Coalho cheese

Efecto del concentrado de proteína de suero y las bacterias endógenas de ácido láctico en el queso Coalho bajo en grasa

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Resumo

O teor de gordura e o fermento láctico são dois componentes que influenciam positivamente as propriedades sensoriais do queijo. No entanto, o consumo de alimentos com baixo teor de gordura é uma tendência entre os consumidores. Nesse contexto, pesquisas sobre aplicações de substitutos de gordura têm sido utilizadas como alternativa para elaboração de queijos com baixo teor de gordura associados à boa aceitação sensorial. Da mesma forma, o uso de fermentos lácticos específicos elaborados a partir de bactérias láticas autóctones é uma alternativa para obter produtos seguros, sem promover mudanças fundamentais em suas características sensoriais. Com o objetivo de avaliar o perfil de textura (Firmeza, Elasticidade, Coesividade e Mastigabilidade) e as características físico-químicas do queijo de Coalho com baixo teor de gordura foram testados o efeito concentrado protéico de soro (1, 2 e 3 %) e das bactérias láticas endógenas (1, 2 e 3%). Os tratamentos do queijo de Coalho produziram queijos classificados com baixo teor de gordura (desnatado) (2, 33% a 4, 67%) e alto valor proteico (41, 01% a 46, 95%). As concentrações de Concentrado Protéico de Soro (CPS) apresentaram efeito significativo ($p < 0,05$) nos parâmetros de firmeza, coesão e mastigação. Conclui-se que o uso de CPS como substituto de gordura mostra-se viável na produção de queijo Coalho. Contudo, a adição de bactérias ácido láticas endógenas como fermento não influencia as propriedades do queijo de Coalho.

Palavras-chave: Queijo regional; Substitutos de gordura; Perfil de textura.

Abstract

The fat content and lactic ferment are two components that positively influence cheese sensorial properties. However, the consumption of low fat content foods is trending among the consumers. Researches about fat replacers applications have been used as an alternative to elaborate low-fat cheeses associated with good sensorial acceptance. In the same way, the use of specific lactic ferments elaborated from lactic acid bacteria isolated from the cheeses themselves is an alternative to obtain safe products, without promoting fundamental changes in its sensorial characteristics. With aim to evaluate the texture profile (Firmness, Elasticity, Cohesiveness and Chewability) and physicochemical characteristics of low-fat Coalho cheese, the effect of whey protein concentrate - WPC (1, 2 and 3%) and endogenous lactic bacteria - ELB (1, 2 and 3%) was tested. The treatments on the Coalho cheese produced cheeses classified as low-fat (2.33% to 4.67%) and high in protein value (41.01% to 46.95%). The WPC concentrations showed significant effect ($p < 0.05$) in firmness, cohesiveness and chewability parameters. In conclusion, the use of the WPC as a fat replacer is viable in the Coalho cheese production. However, the addition of ELB as ferment does not influence the Coalho cheese properties.

Keywords: Regional cheese; Fat replacers; Texture profile

Resumen

El contenido de grasa y la fermento láctico son dos componentes que influyen positivamente en las propiedades sensoriales del queso. Sin embargo, el consumo de alimentos bajos en grasa es una tendencia entre los consumidores. En este contexto, la investigación sobre aplicaciones de sustitutos de grasa se ha utilizado como una alternativa para la producción de quesos bajos en grasa asociados con una buena aceptación sensorial. Asimismo, el uso de fermentos lácticos específicos hechos de bacterias ácido lácticas nativas es una alternativa para obtener productos seguros, sin promover cambios fundamentales en sus características sensoriales. Con el objetivo de evaluar el perfil de textura (firmeza, elasticidad, cohesión y masticabilidad) y las características fisicoquímicas del queso Coalho bajo en grasa fueron probados el efecto del concentrado de proteína de suero (1, 2 y 3%) y de bacterias acido lácticas endógenas (1, 2 y 3%). Los tratamientos de queso Coalho produjeron quesos clasificados como bajos en grasa (desnatados) (2, 33% a 4, 67%) y alto valor proteico (41, 01% a 46, 95%). Las concentraciones de concentrado de proteína de suero (CPS) mostraron un efecto significativo ($p < 0.05$) en los parámetros de firmeza, cohesión y masticación. Se concluye que el uso de CPS como sustituto de grasa resulta ser viable en la producción de queso Coalho. Sin embargo, la adición de bacterias endógenas de ácido láctico para fermentación no influye en las propiedades del queso Coalho.

Palabras clave: Queso regional; Sustitutos de grasa; Perfil de textura

1. Introduction

The Coalho cheese is one of the most traditional cheeses in Brazil, being mostly produced and consumed in the northeast zone of Brazil. This product is obtained through enzymatic coagulation and must present the following sensorial characteristics: semi-hard and elastic, texture, showing compact body with or without small mechanic eyes and a thin rind. Its color is white and uniform, the flavor is mild and slightly acid and can be salty. The fat content of the Coalho cheese is between 35.0% and 60.0% (Brazil, 2001).

The current Brazilian legislation requires Coalho cheese must be made from pasteurized milk. This process ensures food safety, but reduces part of the natural milk microbiota. The raw milk has great microbial diversity, having lots of bacteria that contribute to generate sensorial characteristics on cheese. Specific strains have been isolated from raw milk and added as starter or adjunct cultures, designed to confer the aimed characteristics in products obtained from pasteurised milk. Adding those microorganisms during cheesemaking may compensate the microbial population removal by pasteurization (Quigley et al., 2013).

Allied to the function of lactic ferment in cheese manufacture, fat also plays a fundamental role in sensory quality of food. Fat content varies among cheeses, depending on the milk used and the production method. Even though cheeses usually have high fat content, its usual consumption, together with other dairy products, is recommended, mainly due to the need to reach the daily calcium ingestion recommendation, which is essential to the maintenance of the bone structure (Muniz; Madruga & Araújo, 2013).

Besides that, cheese is also a rich source of other essential nutrients, such as proteins, vitamins, minerals and short chain fatty acids, which can be considered part of a healthy diet (Lópes-Expósito; Amigo & Recio, 2012). Fat also plays an important function at the food taste, appearance and texture. This way, it is evident that the development of low fat content products with high sensorial quality corresponding to its conventional source is a hard task. A technological alternative to play role of ingredient are the fat replacers (Felfoul et al., 2015). Among the fat replacers, proteins have the advantage to bond well to aromatic components, improving the flavor of the low-fat cheeses, as well as its texture.

The purpose of the study was to evaluate the effect of the whey protein concentrate and endogenous lactic bacteria on the physicochemical characteristics and texture profile of a low-fat Coalho cheese.

2. Metodhology

This research was based on a laboratory study. Cheese samples made with whey protein concentrate and endogenous lactic cultures were studied in the research. The results obtained were quantitatives with statistical analysis.

2.1 Obtaining of raw material and experimental design

Skim (< 0.5%) and pasteurized milk used in Coalho cheesemaking was obtained from a production plant in Caucaia, Ceará, Brazil. Two *Lactobacillus* strains, previously isolated from an artisanal Coalho cheese and selected by its technological characteristics, belonging to the Microorganisms Collection of the Brazilian Company of Agricultural Products (Embrapa), were used ELB as lactic ferment in manufacturing cheese: *Lb. rhamnosus* (BRM 029693) and *Lb. plantarum* (BRM 029692). The WPC (Glanbia Nutritionals) used was obtained from a local distributor, composed by 80% of whey protein. Rennet powder (Halamix, Chr. Hansen A/S), calcium chloride (AxaQuímica LTDA) and salt were also used in the Coalho cheese manufacturing.

Factorial experimental design (2^2) was performed with three repetitions of the central point, totalling seven experiments, using two levels (1 and 3 %) of each independent variable - ELB and WPC concentrations. Table 1 shows the experimental design used in this research.

Table 1. Experimental design of the Coalho cheeses with ELB and WPC.

Treatments	Codified variables		Real Variables	
	X_1	**X_2**	**ELB (%)**	**WPC(%)**
1	-1	-1	1	1
2	-1	+1	1	3
3	+1	-1	3	1
4	+1	+1	3	3
5	0	0	2	2
6	0	0	2	2
7	0	0	2	3

X_1 = Endogenous lactic bacteria (ELB) (%) X_2 = Whey Protein Concentrate (WPC) (%)
Source: Elaborated by the authors.

2.2 Endogenous lactic acid Bacteria Growth Conditions

The *Lb. rhamnosus* and *Lb. plantarum* strains were prepared, independently, at the Food Microbiology Laboratory at Embrapa Tropical Agroindustry to constitute ELB. The strains were cultivated in Man, Rogosa, Sharpe medium – MRS (Difco, Sparks, EUA), at 37 ºC for 24 hours and successively peaked for three days, until bacterial concentration of 10^9 CFU/ml. The

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strains were inoculated at Reconstituted Skim Milk (RSM) at 10% (m/v) and incubated at 37 ºC for 24 hours to process the Coalho cheese.

2.3 Cheesemaking

Coalho cheese was produced at the Dairy Laboratory of the Food Engineering Department of the Federal University of Ceará (Fortaleza, Brazil). For each batch it was used the total of 20L of skim pasteurized milk. WPC and the milk were mixed together in a blender in the concentration of 10% (m/v). Both were heated to 40ºC, when ELB was added to ferment at room temperature for 20 minutes. After 70 minutes the addition of calcium chloride ($CaCl_2$) and Rennet powder, the coagulation of milk was observed. Then, the curd was cut, with agitation (three minutes) interrupted by resting periods (five minutes). These procedures were repeated three times. The curds/whey mixture were cooked at 45 ºC for 30 minutes prior to whey drainage. Salting was by means of the application of 2% (w/w) dry salt to the curd pieces, which were formed, pressed for two hours, and stored under freezing at 4ºC.

2.4 Microbiological analysis

The microbiological analyses are performed four days after the cheese processing, at the Food Microbiology Lab at EMBRAPA. The lactic acid bacteria (LAB) counting of each strain source used and of the Coalho cheese samples obtained in the experimental design were performed in *Man Rogosa and Sharpe* (MRS) medium (Difco, Sparks, EUA) through surface plating and incubation at 35 ± 2ºC/ 48 h, according to modified methodology previously described by Hall, Ledenbach and Flowers (2001). The result was expressed in CFU/g.

2.5 Physicochemical analysis

Coalho cheese samples were ground and homogenized. The material was submitted to pH measurement through potentiometric method (Starter 300), titratable acidity in lactic acid, moisture content through gravimetric method, protein content through Micro-Kjeldahl method, fat content through Gerber method using specific butyrometer for cheeses, ash content through muffle furnace (Quimis) under 550ºC, and chloride through Mohr method (IAL, 2008). Water Activity (A_w) analysis was performed by Aqualab equipment (model 4TE).

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The texture profile parameters of firmness (N), elasticity, cohesiveness and chewability (N) test were carried out in the Stable Micro Systems TA-XT2 texture analyzer (Stable Micro Systems). Cylinders of 25 × 20 mm cheeses were cut and the probe (P35) was advanced at 1 mm/sec until it reached a depth of 10 mm for cheese. The penetration force (in g) was recorded after the probe contacted that sample and achieved the minimal trigger force (5 g for cheese). Measures were taken in quadruplicate at room temperature (Oeffner et al., 2013) through the software Exponent Lite Express (version 5.0.9.0 TX Express).

2.6 Statistical Analysis

ANOVA - Variance analysis was used to test differences among seven experimental cheeses treatments (ELB and WPC concentrations) and significance was tested at $p < 0.05$. Tukey test were used to compare the calculated means at 5% of significance level. Data were analyzed using Statistica software version 10.0 (STATSOFT, 2011).

3. Results and Discussion

The highest numbers of viable acid lactic bacteria (10^9 CFU/g) were observed in samples cheese (Table 2), showing that the ELB concentrations used as ferment did not interfere in the growing of the bacteria added in the cheesemaking. Table 2 shows the count of lactic acid bacteria found in Coalho cheese samples.

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Table 2. Lactic Acid Bacteria counting on the endogenous lactic cultures and on the Coalho cheese samples.

Endogenous lactic cultures	Counting (CFU/mL)
Strain 1115	5.0×10^8
Strain 1120	6.5×10^9
Treatments	
T1	1.0×10^9
T2	1.9×10^9
T3	7.0×10^9
T4	1.5×10^9
T5	1.5×10^9
T6	6.0×10^9
T7	2.5×10^9

T1 (ELB 1 %; WPC 3 %); T2 (ELB 3 %; WPC 1 %); T3 (ELB 1 %; WPC 1 %); T4 (ELB 3 %; WPC 3 %); T5 (ELB 2 %; WPC 2 %); T6 (ELB 2 %; WPC 2 %); T7 (ELB 2 %; WPC 2 %). Source: Elaborated by the authors.

The low acidity from the cheeses with ELB and WPC may be due to the increase of the amino acid content in milk by the addition of whey protein concentrate. All the cheeses were classified as medium moisture and fat contents (Brasil, 1996). The cheeses with higher protein content also showed higher moisture, which may be attributed to the bigger bonding capacity with water of proteins. Then, the curd to retain more whey, increasing moisture content (Salama, 2015). The WPC addition was inversely proportional to the protein content, which may be explained by the accelerated proteolysis caused by the addition of lactic bacteria and the higher proteins concentrations. Table 3 shows the physicochemical parameters (acidity, protein, moisture, fat, ash, chloride and Aw) of the Coalho cheese samples.

Table 3. Physicochemical parameters of the Coalho cheese samples.

Treatments	Results (%)						
	Acidity	**Protein**	**Moisture**	**Fat**	**Ash**	**Chloride**	**Aw**
T1	0.41^{ab}	44.38^{b}	42.77^{ce}	4.67^{bc}	2.910^{a}	1.39^{a}	0.9745^{ac}
T2	0.41^{ab}	46.39^{a}	48.20^{ab}	3.00^{cd}	2.932^{a}	1.32^{a}	0.9701^{b}
T3	0.46^{a}	46.95^{a}	49.26^{a}	3.33^{abcd}	2.887^{a}	1.47^{a}	0.9716^{ab}
T4	0.46^{a}	44.21^{bc}	45.98^{bd}	4. 33^{bcd}	2.925^{a}	1.19^{a}	0.9772^{c}
T5	0.41^{ab}	41.83^{d}	44.72^{de}	2.33^{a}	2.941^{a}	1.18^{a}	0.9783^{c}
T6	0.26^{b}	41.90^{d}	44.78^{de}	4.33^{bcd}	2.973^{a}	1.24^{a}	0.9775^{c}
T7	0,26^{b}	41,01^{d}	41,41^{c}	2,67^{ad}	2,887^{a}	1,34^{a}	0,9783^{c}

Data are reported as the Mean. For each physicochemical parameters, means followed by the same letter are not significantly different ($P < 0.05$) by Tukey's test.

T1 (ELB 1 %; WPC 3 %); T2 (ELB 3 %; WPC 1 %); T3 (ELB 1 %; WPC 1 %); T4 (ELB 3 %; WPC 3 %); T5 (ELB 2 %; WPC 2 %); T6 (ELB 2 %; WPC 2 %); T7 (ELB 2 %; WPC 2 %). Source: Elaborated by the authors.

According to the experimental design performed, the parameters of firmness, cohesiveness and chewability were positively affected by the concentrations of WPC, which did not occur with different percentage additions of ELB. The instrumental firmness is defined as the force peak during the compressions first cycle (Bourne, 2002). The structure of the protein matrix has influence over the cheese texture characteristics, so that high protein content is related to high hardness levels (Lobatto-Calleros *et al.*, 2007). In the cheese samples analyzed, the firmness values were between 10.337 N and 27.811 N (table 4). It was observed that the independent variables (ELB and WPC) had distinct effects on the firmness parameter. Only the WPC concentration variation showed significant effect on the performed assay, at 5 % significance level. The increasing on the WPC level contributed directly to the firmness decreasing, which can improve low-fat cheese texture, once these products have higher casein content, which causes stronger firmness and rubbery aspect so as higher chewability. About the elasticity parameter, which consists in the product recovery after compression (Bourne, 2002), the values obtained in the texture profile assays were between 0.828 and 0.850 (Table 4).

Lobato-Calleros et al. (2007) analyzed reduced fat content and WPC added Manchego cheeses, and they did not observe any significant difference to the commercial cheese for the parameters of hardness, cohesiveness, elasticity, and chewability. The cohesiveness consists in the resistance of the internal bonds that composes the product (Gunasekaran; Ak, 2003). The cohesiveness values obtained in the texture profile assays varied between 0,760 and 0,779. The independent variables (WPC and ELB) did not show significant effect (p > 0, 05), only the interaction between WPC and ELB showed significant effect on the performed study. The variable WPC showed significant effect, at 5 % level, according to this parameter. Table 4 shows the means obtained in the texture profile of the Coalho cheese samples obtained by the experimental design.

Table 4. Response variables values from the Coalho cheese experimental design added different ELB and WPC concentrations.

Treatments	Variables (%)		Response			
	ELB	**WPC**	**Firmness (N)**	**Elasticity**	**Cohesiveness**	**Chewability**
T1	1.00	3.00	12.624	0.832	0.762	962.9
T2	3.00	1.00	20.910	0.837	0.760	1588.0
T3	1.00	1.00	27.811	0.850	0.778	2162.8
T4	3.00	3.00	12.687	0.848	0.775	982.7
T5	2.00	2.00	12.503	0.841	0.771	964.1
T6	2.00	2.00	12.873	0.840	0.776	998.1
T7	2.00	2.00	10.337	0.828	0.770	795.8

Data are reported as the Mean.

T1 (ELB 1 %; WPC 3 %); T2 (ELB 3 %; WPC 1 %); T3 (ELB 1 %; WPC 1 %); T4 (ELB 3%; WPC 3 %); T5 (ELB 2 %; WPC 2 %); T6 (ELB 2 %; WPC 2 %); T7 (ELB 2 %; WPC 2 %). Source: Elaborated by the authors.

4. Conclusions

The present work contributes to the improvement of low-fat cheese formulations for restricted diets.

The use of whey protein concentrate as a fat replacer is viable in the Coalho cheese production. The Coalho cheese samples added whey protein concentrate show high protein value and low fat content, being a high nutritional value product.

Beside, the fat replacer decreases firmness and chewability in the texture profile of the low-fat cheeses. The different concentration of endogenous lactic acid bacteria present high counts, but they do not influence the Coalho cheese properties analyzed in this research.

For future work, can study new proportions of whey concentrated applied in manufacture of dairy products, as well as new fat substitutes. In addition to the analyzes in the present research, sensory analysis could be performed to assess potential consumers.

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